



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

In vitro susceptibility of the fish pathogen *Aeromonas salmonicida* to flumequine

Citation for published version:

Barnes, AC, Lewin, CS, Hastings, TS & Amyes, SGB 1991, 'In vitro susceptibility of the fish pathogen *Aeromonas salmonicida* to flumequine', *Antimicrobial Agents and Chemotherapy*, vol. 35, no. 12, pp. 2634-2635. <https://doi.org/10.1128/AAC.35.12.2634>

Digital Object Identifier (DOI):

[10.1128/AAC.35.12.2634](https://doi.org/10.1128/AAC.35.12.2634)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Antimicrobial Agents and Chemotherapy

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



In Vitro Susceptibility of the Fish Pathogen *Aeromonas salmonicida* to Flumequine

A. C. BARNES,^{1*} C. S. LEWIN,¹ T. S. HASTINGS,² AND S. G. B. AMYES¹

Department of Medical Microbiology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG,¹ and DAFS Marine Laboratory, Aberdeen AB9 8DB,² United Kingdom

Received 12 June 1991/Accepted 28 September 1991

The activity of the fluoroquinolone flumequine was investigated against the fish pathogen *Aeromonas salmonicida* and was compared with that of oxolinic acid. Flumequine was more active than oxolinic acid in terms of its MIC against oxolinic acid-resistant isolates of *A. salmonicida* and was as active as oxolinic acid against susceptible isolates. In contrast to oxolinic acid, flumequine was bactericidal, with only 1% of the bacteria surviving 6 h of exposure to the drug at concentrations slightly above the MIC. Mutation to resistance to flumequine was found to occur at a lower frequency than that to oxolinic acid. Hence, in vitro, flumequine appears to possess some advantages over oxolinic acid against this fish pathogen.

Aeromonas salmonicida is the causative agent of furunculosis, an important disease in farmed salmonids in Scotland, Norway, and Canada. The control of furunculosis is dependent upon the use of antibiotics, commonly the 4-quinolone oxolinic acid (1, 4). However, the early success achieved with oxolinic acid is increasingly being compromised by the development of resistance to the drug (5). In a previous study (2), we reported the increased activity of newer fluoroquinolones against *A. salmonicida*. However, it seems unlikely that some of these compounds will become available for veterinary use in the United Kingdom in the near future. In contrast, flumequine is a fluoroquinolone already used in the aquaculture industry in Norway, although it is not used in the United Kingdom. The in vitro activity of flumequine against Scottish isolates of *A. salmonicida* was therefore investigated to assess the potential of flumequine as an alternative to oxolinic acid.

The MICs of oxolinic acid and flumequine were determined for 83 oxolinic acid-susceptible and -resistant isolates of *A. salmonicida*, predominantly from natural outbreaks of furunculosis at farm sites in Scotland. MICs were determined at 22°C by agar dilution on tryptone soy agar (TSA; Oxoid) as described previously (2).

In terms of its MIC, flumequine was slightly more active than oxolinic acid against both oxolinic acid-susceptible and -resistant isolates of *A. salmonicida*, although the range of flumequine MICs was much broader than those recorded for oxolinic acid against resistant isolates (Table 1).

The bactericidal activities of the drugs were investigated essentially as described by Lewin et al. (7) by using two oxolinic acid-resistant isolates, MT364 and MT472, and two oxolinic acid-susceptible isolates, MT363 and MT736 (Table 2). An overnight culture of the isolates containing approximately 10⁸ CFU/ml was diluted 1 in 50 into fresh tryptic soy broth (TSB; Oxoid) containing various concentrations of antibacterial agents. The antibacterial concentrations followed the arithmetic progression 1.5, 3, 5, and 9. Samples were taken after 1, 3, and 6 h of incubation at 22°C and were serially diluted in TSB. Percent survival was estimated by viable count on TSA. Experiments were performed in triplicate, and mean values are plotted in Fig. 1.

TABLE 1. Range of MICs of flumequine and oxolinic acid against 83 Scottish isolates of *A. salmonicida*

Strain type (no. of isolates)	Test agent	MIC (μg/ml) at 22°C ^a		
		50%	90%	Range
Oxolinic acid resistant (38)	Oxolinic acid	3.00	7.50	1.00-15.00
	Flumequine	2.00	4.00	0.10-20.00
Oxolinic acid susceptible (45)	Oxolinic acid	0.030	0.40	0.01-0.75
	Flumequine	0.075	0.10	0.075-0.50

^a 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

After 1 h of exposure, neither oxolinic acid nor flumequine was bactericidal, with approximately 100% of the bacteria surviving at concentrations of up to 100 times the MIC for the bacteria (data not shown). Furthermore, oxolinic acid was merely bacteriostatic even after 6 h of exposure, with approximately 100% of the bacteria surviving (Fig. 1). In contrast, flumequine was bactericidal, killing 90% and >99% of the bacteria after 3 and 6 h of exposure, respectively, to concentrations above five times the MIC at 22°C (Fig. 1). The bactericidal activity of flumequine may be of use in eliminating the asymptomatic carrier state of furunculosis;

TABLE 2. MICs of oxolinic acid and flumequine for isolates used in bactericidal activity and mutation frequency studies

Isolate ^a	MIC (μg/ml)	
	Oxolinic acid	Flumequine
MT363 ^b	0.03	0.10
MT736	0.015	0.075
MT364	7.50	2.00
MT472	3.00	3.00
MT490 ^b	0.03	0.10
MT494 ^b	0.03	0.10
MT744 ^b	0.03	0.075
MT747 ^b	0.03	0.10

^a All strains were isolated from separate outbreaks of furunculosis in Scotland.

^b Strains used in the mutation frequency study.

* Corresponding author.

TABLE 3. Range of mutation frequencies for five oxolinic acid-susceptible isolates of *A. salmonicida*

Test agent	Mutation frequency at:		
	5× MIC	10× MIC	20× MIC
Oxolinic acid	3.3×10^{-9} – 3.64×10^{-8}	1.7×10^{-9} – 6.4×10^{-8}	$<1.1 \times 10^{-9}$ – 3.2×10^{-8}
Flumequine	1.5×10^{-9} – 2.0×10^{-8}	$<3.3 \times 10^{-10}$	$<3.33 \times 10^{-10}$

indeed, Scallan and Smith (8) obtained promising results with flumequine in reducing carriers in salmon and trout.

Resistance to antibacterial agents is a serious problem in the aquaculture industry (5, 11), and the effective life of an antibiotic is determined largely by the rate at which resistance to it develops during therapy. Determination of the frequency of chromosomal mutation that leads to resistance can provide an indication of the rate at which resistance to a quinolone will develop (9), since plasmid-mediated resistance to the quinolones has not yet been identified clinically (3). Mutation frequencies were determined for five oxolinic acid- and flumequine-susceptible isolates of *A. salmonicida*, as follows. Conical flasks containing 250 ml of drug-free TSB were inoculated with the *A. salmonicida* isolates and were shaken overnight at 22°C. The resulting cultures were centrifuged at $11,500 \times g$ and resuspended in 2 ml of fresh TSB to give cell concentrations in excess of 10^{10} CFU/ml. Aliquots (0.1 ml) of undiluted and 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4} dilutions of the cultures were then spread onto TSA plates containing antibiotic at 5, 10, and 20 times the MIC. The plates were incubated at 22°C and were examined daily for 7 days for the presence of resistant colonies. Resistant colonies were subcultured onto TSA containing antibiotic to verify resistance, and colonies were identified to the species

level by using a latex bead agglutination kit (Aquaculture Vaccines Ltd., Saffron Walden, United Kingdom).

A. salmonicida isolates developed resistance to flumequine at significantly lower frequencies than they did to oxolinic acid (Table 3). Indeed, resistance to flumequine developed at less than 1/10th the frequency that resistance to oxolinic acid did.

In conclusion, flumequine was only marginally more active than oxolinic acid in terms of its MIC. However, flumequine was bactericidal at concentrations slightly above the MIC after 6 h of exposure, whereas oxolinic acid was merely bacteriostatic. This bactericidal activity, combined with the lower mutation frequencies and better pharmacokinetics (6, 10), suggest that flumequine has several advantages as a potential alternative to oxolinic acid for use in Scotland. This is supported by the encouraging results achieved with flumequine during furunculosis therapy in Norway.

We thank the Science and Engineering Research Council for the CASE studentship to A.C.B. and the Scottish Salmon Growers Association for financial support of the project.

REFERENCES

1. Austin, B., J. Rayment, and D. J. Alderman. 1983. Control of furunculosis by oxolinic acid. *Aquaculture* 31:101–108.
2. Barnes, A. C., C. S. Lewin, T. S. Hastings, and S. G. B. Amyes. 1990. *In vitro* activities of 4-quinolones against the fish pathogen *Aeromonas salmonicida*. *Antimicrob. Agents Chemother.* 34:1819–1820.
3. Courvalin, P. 1990. Plasmid mediated 4-quinolone resistance—a real or apparent absence? *Antimicrob. Agents Chemother.* 34:681–684.
4. Endo, T., K. Ogishima, H. Hayasaka, S. Kaneko, and S. Oshima. 1973. Application of oxolinic acid as a chemotherapeutic agent against infectious diseases in fish. *Bull. Jpn. Soc. Sci. Fish.* 39:165–171.
5. Hastings, T. S., and A. McKay. 1987. Resistance of *Aeromonas salmonicida* to oxolinic acid. *Aquaculture* 61:165–171.
6. Hustvedt, S. O., and R. Salte. 1991. Pharmacokinetics in farmed Atlantic salmon. In C. Michel and D. J. Alderman (ed.), *Problems of chemotherapy in aquaculture: from theory to reality*. Publ. Office International des Epizooties, Paris.
7. Lewin, C. S., B. M. A. Howard, N. T. Ratcliffe, and J. T. Smith. 1989. 4-Quinolones and the SOS response. *J. Med. Microbiol.* 29:139–144.
8. Scallan, A., and P. R. Smith. 1985. Control of asymptomatic carriage of *Aeromonas salmonicida* in Atlantic salmon smolts with flumequine. In A. E. Ellis (ed.), *Fish and shellfish pathology*. Academic Press Ltd., London.
9. Smith, J. T. 1984. Mutational resistance to 4-quinolone antibacterial agents. *Eur. J. Clin. Microbiol.* 3:347–350.
10. Syvertsen, C., A. Rogstad, and G. Berge. 1989. Aqualets, a drug delivery system for farmed fish. In *Diseases of fish and shellfish*. IV EAFP International Conference, Santiago, Spain. European Association of Fish Pathology, Aberdeen, United Kingdom.
11. Tsoumas, A., D. J. Alderman, and C. Rodgers. 1989. *Aeromonas salmonicida*; development of resistance to 4-quinolone antimicrobials. *J. Fish Dis.* 12:493–507.

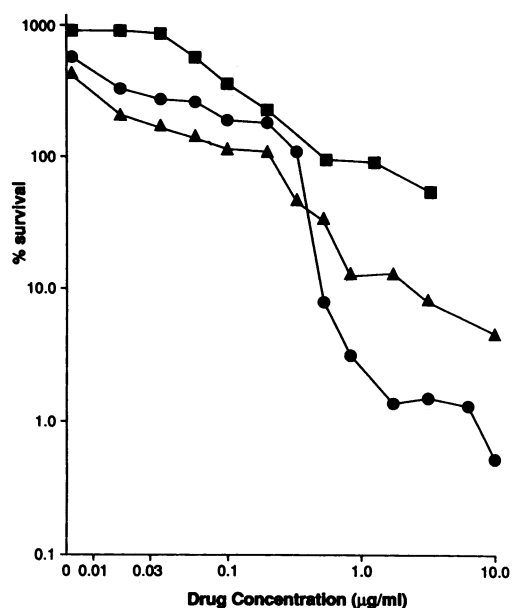


FIG. 1. Bactericidal activities of oxolinic acid and flumequine against *A. salmonicida* MT363. Percent survival after 6 h of exposure to oxolinic acid (■), 3 h of exposure to flumequine (▲), and 6 h of exposure to flumequine (●) is shown.